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Journal of Cystic Fibrosis 10 (2011) 143 – 144

Journal of Cystic Fibrosis

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Letter to the Editor

Optimizing strategies for *CFTR* molecular testing

Sir,

The wide mutational spectrum found in cystic fibrosis (CF; OMIM 219700; http://www.genet.sickkids.on.ca) is a permanent challenge that needs specific approaches in each population. Currently, *CFTR* genetic testing is performed applying commercial panels and/or sequencing (directly or after scanning techniques) [1]. However, once the mutation has been characterized, a specific method is usually developed (heteroduplex analysis, Taqman probe, RFLP, etc.) to facilitate its detection in further studies concerning a family and/or a particular population.

Among 250 mutations characterized in the Spanish CF population [2,3] three have been found close together in exon 10, p.Phe508del (F508del; 52%), p.Ile507del (I507del; 1%) and c.1545_1546delTA (1677delTA; 0.1%). In addition to patients/families bearing one of these mutations, we have identified nine compound heterozygous patients (6 p.Phe508del/p.Ile507del; 2 p.Phe508del/c.1545_1546delTA; and 1 p.Ile507del/c.1545_1546delTA).

A previous protocol [4] was modified to offer cascade carrier testing. Briefly, the PCR conditions were, denaturation (94° C/3 min), 30 cycles 94 °C/20 s, 62 °C/30 s, 74 °C/30 s and extension (74 °C/5 min) followed by electrophoresis on an 8% polyacrylamide gel (PAGE; 20 × 20cm) for 4 h at 160 V and ethidium bromide staining.

Nine DNAs from different sources were analysed to evaluate whether the patterns in heterozygous, homozygous and compound heterozygous individuals could be discriminated. The different genotypes differed by 0–3 base pairs (Fig. 1). Homozygous p.Phe508del, homozygous p.Ile507del and compound heterozygous p.Phe508del/p.Ile507del (# 1,3,5) showed the same profile as both mutations determine the deletion of three base pairs and in this case the heteroduplex bands were indistinguishable from the homoduplex. Among the other samples, six specific patterns were observed, one wild type, three heterozygous samples (# 4,7,8) and 2 compound heterozygous (# 2,6). Interestingly, visualization of the heteroduplex bands was found to be as important as homoduplex for assigning each genotype.

Given the specific mutation patterns, the analysis was applied to a high risk couple who requested a prenatal diagnosis. DNAs were extracted from blood leukocytes (parents), dried blood spot (index case with genotype p.Phe508del/c.1545_1546delTA) and the chorionic villus sample. PCR products were detected in all the samples. The foetal and the paternal samples showed the c.1545_1546delTA heterozygous pattern. As recommended [1], maternal contamination was discarded analysing two microsatellite markers, IVS8CA and IVS17bTA (data not shown).

Maximizing test efficiency in target populations

The c.1545_1546delTA reaches high frequencies in specific populations. Particularly, it has been reported in Russia (0.8%) [5], Greece (0.9%) [6], Bulgaria (2.4%) [7] and Turkey (7.2%) [8]. In contrast, the p.Ile507del mutation has not been detected either in Bulgaria or in Turkey. Overall, the analysis of the three mutations accounts for 30.7% of CF alleles in Turkey, 55.0% in Greece and Russia and 67.8% in Bulgaria.

For these mutations, discrepancies and misinterpretation of genotypes have been reported for commercial panels [9].

Here, we propose an easy method for the suitable detection of three nearby CF-causing mutations facilitating the CF molecular diagnosis in those populations in which the mutations are prevalent and reducing the number of samples for further more expensive mutation panels. For the three undistinguished patterns, the analysis of the CF parents would be determinant as each mutation to be detected shows a specific pattern in

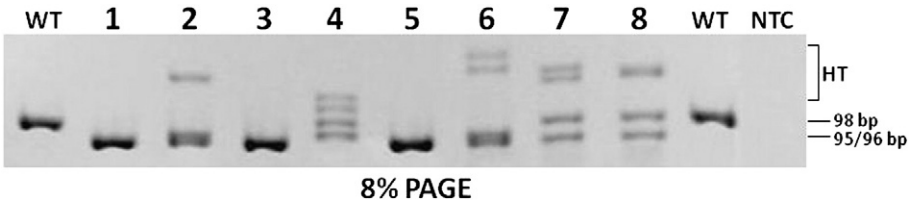

Fig. 1. Direct analysis of three CF-causing mutations in exon 10. PCR primers: C16B, 5′-GTT TTC CTG GAT TAT GCC TGG CAC-3′; C16D, 5′-GTT GGC ATG CTT TGA TGA CGC TTC-3′ [4]. WT, wild type; NTC, no template control; HT, heteroduplex; PAGE, polyacrylamide gel electrophoresis. Samples: 1, p.Phe508del/p.Phe508del; 2, p.Phe508del/c.1545_1546delTA (1677delTA); 3, p.Ile507del/p.Ile507del; 4, c.1545_1546delTA/wt; 5, p.Phe508del/p.Ile507del; 6, p.Ile507del/c.1545_1546delTA; 7, p.Phe508del/wt; and 8, p.Ile507del/wt.

doi:10.1016/j.jcf.2010.11.002

heterozygous samples. This accurate analysis could be applied to the cascade carrier testing and prenatal diagnosis, as well as in the flowchart for CF diagnostic testing in particular populations. Furthermore, the strategy might be advantageous in other genetic disorders showing mutations with similar characteristics. In summary, we propose joining old and new technologies to achieve a rational optimization of the available resources.

Acknowledgements

Supported by grants from ISCiii FIS/FEDER PI080041 and Fundación Sira Carrasco (Spain).

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